ENVIRONMENTAL FACTORS AFFECTING THE RESISTANCE OF CERTAIN AGROTRICUMS AND THEIR DERIVATIVES AGAINST WHEAT STREAK MOSAIC

BY

SHEN-THEN CHANG

Bachelor of Science in Agriculture
National Taiwan University
Taipei, Taiwan, Republic of China
1974

Master of Science
University of Idaho
Moscow, Idaho
1979

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
July, 1984

ENVIRONMENTAL FACTORS AFFECTING THE RESISTANCE OF CERTAIN AGROTRICUMS AND THEIR DERIVATIVES AGAINST WHEAT STREAK MOSAIC

Thesis Approved:

Francis O. Blough
Thesis Adviser

H. C. Young Jr. / by F. O. Blough

L. K. Simpson

D. E. Wadsworth

E. L. Smith

Norman N. Durham
Dean of Graduate College

ACKNOWLEDGMENTS

I am deeply indebted to my retired major adviser, Dr. H. C. Young, Jr., for his guidance, assistance, and encouragement through this study and preparation of manuscript. I also wish to express my great gratitude to my thesis adviser, Dr. F. J. Gough, for his guidance after Dr. Young retired. I also wish to express my great appreciation to other committee members: Dr. D. F. Wadsworth, Dr. L. L. Singleton, and Dr. E. L. Smith, for critical review and suggestion concerning my study and manuscript preparation.

I wish to thank the Department of Plant Pathology of Oklahoma State University for providing the facilities and financial support during the study.

Finally, I wish to express my gratitude to my parents, Mr. and Mrs. Chan-Chi Chang, my brother and sisters, and my wife, for their love, patience, and encouragement while I was pursuing higher education.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure | Page

CHAPTER I

INTRODUCTION

Wheat (Triticum aestivum L. em Thell) is the most widely grown grain crop in the world. Its grain is a major food source for both mankind and livestock. In certain areas where the winter is mild, winter wheat is used also as winter pasture for cattle, which often contributes as much cash value to income as the grain harvest. Associated with extensive cultivation of wheat are various disease problems, among which is the wheat streak mosaic virus disease (WSM).

Symptoms of WSM on susceptible wheat first appear as light green mottling of newly expanding leaves, followed by light-colored streaks along leaf veins and a general stunting of plants. As the disease progresses, leaves become yellowish green with marked yellow mottling and striping. Severely affected plants may be very light waxy yellow in color. Infected plants may also appear more prostrate (47).

Wheat streak mosaic disease is caused by wheat streak mosaic virus (WSMV). Since its discovery by McKinney (20), WSMV has been identified in most central and western states of the U.S. WSMV has a particle size of 15 x 700 nm (12), with a single strand RNA core and a protein coat. This virus has characteristics that classify it between potato virus S and potato virus Y (2). WSMV can be mechanically transmitted without difficulty.

In the field, WSMV is transmitted by its natural vector, the wheat

curl mite Aceria tulipae Keifer (42). The dispersal of WSMV and consequently the spread of WSM depend on the movement of mites by wind (43). WSMV in the field survives from one wheat crop to another in wild native grasses but epidemics of WSM usually only occur when wheat is planted early and volunteer wheat is present in abundance (44,47).

Many control measures have been suggested to combat WSM. Removing volunteer wheat and delaying the seeding date have been very successful in reducing the incidence of WSM. However, in areas like Oklahoma where early planting is widely adopted to promote foliar growth of winter wheat for winter pasture, and minimum tillage is suggested for soil erosion control, WSM can be controlled effectively and economically only through the utilization of resistant wheat cultivars. Unfortunately, almost all wheat, both winter and spring types, are susceptible to WSM (3). There is some evidence that non-specific resistance to WSM exists in wheat, but no progress in breeding for this type of resistance has been made (38). There are some wheat cultivars that do exhibit a certain degree of tolerance to WSM at least under some condition. Compared to the yield of non-infected wheat, however, the yield of WSM-infected tolerant cultivars are not acceptible economically.

Many plant species, such as Secale and Agropyron spp., taxanomically closely related to wheat show resistance to WSM (23). Extensive efforts have been made to transfer resistance from Agropyron spp. to wheat. C.I. 15321[1/], a disomic substitution line, and C.I. 15322, a disomic translocation line, developed by E. E. Sebesta in Oklahoma, both show resistance to WSM. In both lines the resistance was derived

1/ C.I. numbers are assigned by the Germplasm Resource Laboratory, ARS, U.S. Department of Agriculture, Beltsville, Md.

from Agropyron elongatum (36). However, they have not been used successfully in the wheat breeding program due to their low yield potential and the difficulty of transferring the resistance into high yield wheat lines.

In controlled environments, plants of C.I. 15321 and C.I. 15322 occasionally show systemic symptoms of WSMV infection. Since it is known that many environmental factors affect the symptom expression of many viral diseases, it seemed advisable to attempt to recognize factors that alter the resistance and /or symptom expression of these two WSM resistant lines.

In this study, the effects of temperature, fertilizer level, and photoperiod on the expression of resistance of C.I. 15321, C.I. 15322 and OK 9387A were investigated. The inheritance of resistance in OK 9387A (derived from C.I. 15322) and temperature effect on phenotypic expression of this resistance in a segregating F_2 population also were studied.

CHAPTER II

LITERATURE REVIEW

Susceptibility of plants to virus infection and symptom development of viral diseases are known to be influenced by environmental factors such as temperature, light intensity, photoperiod, plant nutrition, and so on. Among those factors temperature probably has the most profound effect. Temperature effects can be divided into pre-inoculation effects on host predisposition and post-inoculation effects.

Post-inoculation Temperature Effects

Johnson (16) showed that within the temperature range of 28-30 C inoculated tobacco plants (Nicotiana tobaccum) expressed tobacco mosaic symptoms more quickly and more severely than those maintained at a lower temperature. He found systemic mosaic symptoms would be masked if the post-inoculation temperature was higher than 36-37 C. Tobacco mosaic virus, however, was present in inoculated plants as shown by the appearance of mosaic symptoms when these same inoculated plants were again subjected to a temperature favorable for mosaic symptom development. For the potato mosaic virus on potatoes, disease symptoms appeared at post-inoculation temperatures ranging from 6 C to 18 C. The most severe symptoms occurred at temperatures between 14 and 18 C. When temperature was above 20 C, mosaic symptoms were masked. Again, such temperatures did not eliminate potato mosaic virus from infected plants

or tubers even after exposure to 36 C for 10 days (17).

According to Sameul (34), tobacco mosaic virus in Nicotiana glutinosa induces necrotic spots of 1-2 mm in diameter at 21 C, but at 28 C post-inoculation temperature much larger and more faster spreading necrotic spots develop. At 35 C, no necrotic spots formed and inoculated leaves show only faint yellow blotchs as primary lesions. At 21 and 28 C, when necrotic local lesions are formed, systemic infection of the plants does not occur; whereas, at the high temperature of 35 C when no necrosis is produced on the inoculated leaves systemic infection of the plants occurs rapidly. It has been shown that N. glutinosa inoculated with tobacco mosaic virus and kept at 35 C would collapse and die in one day after being transferred to 21 C. Not all virus - host combinations that give local lesions at low temperatures result in systemic infection of virus at high temperatures. Bean and cucumber genotypes that become locally infected with tobacco mosaic virus behave the same at both 20 and 36 C (22).

Stevenson and Rand (49) investigated the post-inoculation temperature effects on symptom expression of pea seed-borne mosaic virus infected peas. They concluded that symptom progression on the susceptible cultivars Alaska and Dark Skin Perfection was faster at high temperature than at low temperature, but the final severity of symptoms seemed not to be affected significantly by temperature.

The foliar symptom expression of prune dwarf in susceptible prune was reported to be completely masked when plants were grown at 22 C or higher after inoculation, but developed typically when plants were grown at 13 C post-inoculation temperature (25).

Stingl and King (50) demonstrated that mild mottle symptoms on

Fragaria vesca were masked at 26 C post-inoculation temperature while the most pronounced symptoms developed at 16 C post-inoculation temperature. Fragaria virginiana was a symptomless carrier of mild mottle virus at all temperature regimes. Veinbanding virus induced a cyclic appearance of symptoms on F. vesca at all temperature regimes. This virus produced severe symptoms on F. virginiana at 26 C post-inoculation temperature, but this host remained symptomless at 16 C post-inoculation temperature.

Diachum (8) reported that tobacco streak virus produced discrete small necrotic spots on inoculated tobacco leaves at 20 C post-inoculation temperature, but produced large necrotic arcs, broken rings, and line and dot patterns when plants were incubated at 30 C after inoculation. At intermediate post-inoculation temperature regimes, concentric rings were produced from tobacco streak virus infection.

Gill and Westdal (11) studied the effect of post-inoculation temperature on symptom severity of barley infected with aster yellows or barley yellow dwarf virus. Aster yellows symptoms were very severe at 32 C, mild at 21 C, and absent at 16 C. The temperature effect was reversed with barley yellow dwarf. Symptoms were masked at 32 C, very mild at 27 C, and severe at 16 C.

Foster and Webb (10) reported that with watermelon mosaic virus 1, watermelon mosaic virus 2, cucumber mosaic virus, squash mosaic virus, and muskmelon necrotic fleck virus, the severity of symptoms on muskmelon decreased as the post-inoculation temperature increased.

Singh et al. (39) tested the effect of post-inoculation temperature on disease development and seed transmission of barley stripe mosaic virus on wheat, barley, and corn at 16, 20, 24, and 28 C. Symptom

severity increased with an increase of post-inoculation temperature on all the crop plants tested. The effect of soil temperature on symptom severity was less marked than was air temperature. The rate of seed transmission of barley stripe mosaic virus was highest also for barley plants incubated at high post-inoculation temperatures.

Schneider and Worley (35) reported that higher post-inoculation temperature increased the susceptibility of detached bean leaves to southern bean mosaic virus infection. They found not only an increase in the number of local lesions but also an increase in lesion size.

Sill (41) examined post-inoculation air temperature effects on symptomatology of wheat streak mosaic on five wheat cultivars at 16, 20, 24, and 28 C, all grown in 8 C soil temperature. The incubation period was always shortest at higher temperatures, and symptom severity was greatest. At 16 C air temperature, two cultivars failed to develop systemic symptoms two months after inoculation although wheat streak mosaic virus was still present in the plants.

Martin (19) reported that the rate of systemic symptom development of wheat streak mosaic on the tolerant wheat cultivar Eagle did not differ from that on the susceptible cultivar Parker. Both cultivars developed systemic symptoms more slowly at lower post-inoculation temperature than at higher post-inoculation temperature.

Pre-inoculation Temperature Effects

Using the increase in number of local lesions as a measure of susceptibility of plants to virus infection, Kassanis (18) found that as little as six hours at a temperature of 36 C prior to inoculation increased susceptibility of beans to tobacco necrosis virus infection.

The same effect was noted with N. glutinosa and the tobacco mosaic virus and the tomato bushy stunt virus, and with tobacco and the tomato spotted wilt virus. Maximum susceptibility was reached usually after incubating plants for two days at 36 C prior to inoculation.

Yarwood (52) found that heating the primary leaves of beans for 60 seconds in water at 45 C before inoculation increased the number of local lesions induced by tobacco mosaic virus, by tomato spotted wilt virus, and by peach yellow bud mosaic virus; the increase in local lesions was 7 fold, 2.3 fold, and 6.2 fold, respectively. Similar predisposition temperature effects were observed on sugarcane cuttings to sugarcane mosaic virus. The increased susceptibility in the sugarcane cuttings did not carry over into first progeny of the treated canes (53).

Panzer (26) reported bean plants to be more susceptible to alfalfa mosaic virus infection when treated for two hours at 20-30 C before inoculation than when treated at either higher or lower temperatures. With tobacco mosaic virus, however, susceptibility increased only when plants were treated at high pre-inoculation temperatures.

Ganzalaz and Pound (13) presented evidence that both pre- and post-inoculation temperatures could affect the susceptibility of N. glutinosa to infection by cabbage virus A. Plants predisposed at 28 C always had higher numbers of local lesions than plants predisposed at 20 C, when the post-inoculation temperature was the same. However, plants predisposed at lower temperatures would produce more local lesions when maintained under high post-inoculation temperatures than when maintained under low post-inoculation temperatures.

Host Nutrition Effects

Spencer (45) reported that maximum susceptibility of N. glutinosa plants to tobacco mosaic virus was reached at a relative low nitrogen level (15 mg per day) and was not correlated with rapidity of host growth. Maximum susceptibility of bean to tobacco mosaic virus was reached at nitrogen levels of 80-100 mg per day applied as calcium nitrate, whereas maximum growth of host plants occurred at levels of 20-60 mg per day. In the tobacco - tobacco mosaic virus 6 combination, maximum growth of plants occurred at 15 mg of calcium nitrate per day but maximum susceptibility was reached only when 75 mg of calcium nitrate per day was applied.

Spencer (46) also reported that susceptibility of tobacco plants to the infection of tobacco mosaic virus 6 was reduced when more than 20 mg of mono-ammonia phosphate was applied daily while the maximum plant growth occurred throughout a range of 5-80 mg of mono-ammonia phosphate per day. Susceptibility was enhanced by potassium until a level of 20 mg of potassium sulfate per day was reached, then there was a steady decline in susceptibility.

Bawden and Kassanis (1) examined the effect of nutrition on host susceptibility and concluded that increased susceptibility due to phosphate and nitrogen when tobacco was infected with aucuba mosaic virus and when N. glutinosa was infected with tobacco mosaic virus was indirect - simply an increased plant size.

Helms and Pound (14) reported that increased nitrogen levels produced more marked effects on symptom expression, compared to different levels of phosphorus and different levels of balanced nutrients.

In cucumber - tobacco ring spot virus, tobacco - potato virus X, and N. blutinosa - potato virus X combinations, the mildest symptoms always developed at the lowest nitrogen level. Severity of symptoms increased when the nitrogen concentration increased. A nitrogen level of 1,050 ppm caused stunting and/or death of plants. Mild symptoms were related to low phosphorus levels one week after inoculation but symptoms on plants at all phosphorus levels were similar in term of severity two weeks after inoculation. With balanced nutrients, symptoms were milder at high (3 X) or low (0.5 X) concentrations than at intermediate concentrations (1 X and 2 X).

Weathers and Pound (51) reported that severity of tobacco mosaic symptoms on tobacco always increased when nitrogen levels increased. At the highest nitrogen level (1,050 ppm), very large dark green islands developed on distorted and crinkled leaves. Phosphorus levels had little affect on severity of symptoms. With a balanced nutrition, systemic symptoms were most severe at 1 X and mildest at 3 X. Symptoms at 2 X and 0.5 X were slightly less severe than that at 1 X. Pound and Weathers (33) working with turnip virus 1 - N. glutinosa and - N. multivalvis combinations reached the same conclusions except that at the highest nitrogen level (1,050 ppm) their plants showed very mild systemic symptoms instead of severe symptoms.

Foster (9) studied the effect of nutrition on susceptibility of Chenopodium amaranticolor to infection by cucumber mosaic virus. He concluded that high phosphorus, low potassium, and high magnesium concentrations all increased susceptibility. Both high and low levels of nitrogen, low phosphorus, high potassium, low calcium, and low magnesium concentrations reduced susceptibility.

Temperature Effects on Expression of Resistance

Pound and Cheo (30) reported that the spinach cultivar Virginia Savoy was completely resistant to cucumber mosaic virus at 16 and 20 C. At 24 C, inoculated plants developed a very slight mottling and chlorosis with ultimate necrosis of lower leaves, but plants were never severely affected. At 28 C, however, plants developed top necrosis that soon extended downward and killed the plants within seven days. The resistance would persist if plants were exposed at 28 C no longer than 12 hours. The percentage of top necrosis and the rate at which it developed were not greatly affected by any predisposition temperature treatment. Soil temperature had no effect. Although host resistance was not expressed at 28 C, virus multiplication was not detectable by local lesion assay.

Pound (29) studied the relation of air temperature to cabbage mosaic resistance in cabbage. He found that resistant cabbage genotypes expressed resistance only when post-inoculation temperatures were 24 C or lower. Severe symptoms developed on resistant types incubated at 28 C. In this case, the mosaic virus reached high concentrations at high temperatures in the resistant plants.

Bean cultivars normally resistant to common bean mosaic developed veinal necrosis instead of mosaic expressed by susceptible cultivars at 32 C or higher, and the plants died rapidly (33). Necrotic symptoms could be produced easily by exposing resistant plants for four hours to 32 C, then to 20 C for the remaining 20 hours during each day after inoculation. If plants were kept at 16 C for 4 days or longer immediately after inoculation, the symptoms on resistant plants were greatly

reduced.

Carroll and Kosuge (4) found that tobacco variety Xanthi-nc, normally a local lesion host of tobacco mosaic virus, developed chlorotic lesions on inoculated leaves with vein clearing and chlorosis in young apical leaves when maintained at 37 C. Tissues with symptoms became necrotic rapidly after temperature was reduced from 37 C to 22 C. This type of reaction was similar to the reaction of N. glutinosa to tobacco mosaic virus infection at high temperature (34).

Silbernagel and Jafri (40) found that resistance to curly top virus in snap bean cultivar Goldcrop was not expressed if viruliferous leaf hopper-inoculated plants were exposed to 31-34 C post-inoculation temperatures. At lower temperatures, 21-24 C, resistance of Goldcrop was effective. Also, resistance was not related to effectiveness of virus transmission by leaf hopper at different temperatures.

Martin (19) reported that resistance to WSM in C.I. 15322, a translocation selection of wheat X Agropyron elongatum, was affected by post-inoculation temperature. At 22 C, C.I. 15322 developed no systemic symptoms, but a high percentage (92%) of inoculated plants developed systemic symptoms when incubated at 27 C post-inoculation temperature. Conversely, Pfannenstiel and Niblett (27) found that C.I. 15322 and C.I. 15321 (the latter a substitution line from the same wheat X A. elongatum cross as C.I. 15322) were susceptible to WSM, at least to a certain degree, when inoculated plants were maintained at 18, 27, and 35 C post-inoculation temperatures. They also found that C.I. 15092, a substitution line derived from 'Carsten' wheat x A. intermedium, was resistant to WSM at 18 and 28 C but developed systemic symptoms at 35 C.

Cirulli and Alexander (7) studied the influence of post-inoculation temperature on resistance in a tomato breeding line, 801, which obtained its resistance to tobacco mosaic virus from Lycopersicon peruvianum. An F_2 of 801 X a susceptible tomato line segregated 3 resistant plants : 1 susceptible, and the backcross segregated 1 resistant : 1 susceptible at 15-17 post-inoculation temperature. At 26-28 C post-inoculation temperature, the same F_2 population segregated into 1 resistant: 2 necrotic : 1 susceptible, and the backcross segregated into 1 necrotic : 1 susceptible. They concluded that resistance to tobacco mosaic in 801 was probably controlled by a single dominant gene with possibly a single modifier gene. It also indicated that temperature, along with genetic background of host, may affect the reaction of plants to virus infection.

CHAPTER III

MATERIALS AND METHODS

Source, Preparation, and Inoculation of WSMV

Wheat streak mosaic virus (Marmor virgatum McK. var. typicum McKinney) strain 'Salina' obtained from Dr. E. E. Sebesta, U.S.D.A., A.R.S., Department of Agronomy, Oklahoma State University, Stillwater, was used in all experiments. Dried, refrigerated virus-containing wheat leaves was extracted with tap water and Celite was added as abrassive. The extract was manually inoculated on to the wheat cultivar Blue Jacket (C.I. 12502), grown under greenhouse conditions. Later, these plants were used as a source of inoculum after typical streak mosaic symptoms were developed on new leaves. To prepare the inoculum, plant leaves showing systemic symptoms of WSM were clipped into sections Of 0.5 cm long. To every 100 gm of such leaf tissue 1.5 liter of tap water was added together with 50 gm of Celite abrassive. This mixture was then ground in a Waring blender at speeds from low through intermediate to high for 30 seconds each. The resulting suspension was strained through three layers of cheesecloth to remove inert plant fibers. The final extract was retained in a one liter glass container. An artist's spray paint nozzle (Devilbiss Model EGA-502 GUN) was used for inoculation and was driven by air pressure at 4.9-7 kg/cm^2. Inoculation was accomplished by holding the nozzle about 1 cm from the leaf

to be inoculated until water soaked lesions appeared.

Experiment I: Post-inoculation Temperature Effects

The purpose of the experiment was to determine post-inoculation temperature effects on susceptibility of certain wheats to WSMV infection and to examine possible genotype x temperature interactions. The wheat cultivars used were: Wichita (C.I. 11952), a very susceptible host; Blue Jacket, a tolerant host; the selected wheat lines C.I. 15321, a WSM resistant substitution line selected from the cross Triticum species /A. elongatum//Arlando/Triticum timopheevi/3/Hope/Baart/4/Nebred, with WSM resistance from A. elongatum; C.I. 15322, a WSM resistant translocation line selected from the same cross; and OK 9387A, a WSM resistant selection from the cross 2*Osage/C.I. 15322. Fifteen seeds were sown per 10 cm pot and plants grown in a greenhouse for 4 wk before being inoculated with WSMV. Immediately after inoculation, the plants were transferred to growth chamber (Percival Model PGC 78 C) with temperatures set at 20, 25, 30, and 35 $\pm$ 1 C. Light intensity of 10,550 lx with 12 hr photoperiod was supplied with a thermal barrier between the light source and experimental plants. Two pots of inoculated plants and one of uninoculated check plants were used with each cultivar or line. The experimental design was a split plot with post-inoculation temperature as the main unit and cultivars or lines as subunits. The experiment was conducted three times, each time being treated as a block. Both temperature settings and position of cultivars and lines inside the growth chambers were randomized. The first block was grown from May 18 to June 1, the second from June 9 to June 23, and the third from July 3 to July 17, 1981.

The number of plants in each pot that developed systemic symptoms of WSM was recorded 6 days after inoculation and then every other day until 14 days after inoculation. Susceptibility and resistance of plants within cultivars or lines were based on the development of systemic WSM symptoms. Since plants inoculated and maintained at high temperature regimes died, whether with or without systemic symptoms before final readings and uninoculated check plants at the same temperature regimes did not, the dead plants were classified as plants with systemic symptoms.

Experiment II: Fertilizer and Post-inoculation Temperature Effects

The purpose of this experiment was to determine the effects of fertilizer levels and post-inoculation temperatures on susceptibility of certain wheat cultivars and lines to WSMV infection. The same cultivars and lines used in experiment I were used. Four week old plants were inoculated with WSMV as previously described. The plants were then moved into growth chambers at temperatures of 20, 25, and 30 C; three pots of each cultivar or line per temperature regime. One pot of one cultivar or line at each temperature was supplied with 1, 4, or 7 gm of a granular fertilizer, Nurish (W. R. Grace & Co. Agricultural Chemicals Group; N : P : K ratio of 10 : 20 : 10). Resistance or susceptibility was measured as in previous experiment.

This experiment was made twice and treated as two block. The experimental design was a split plot with post-inoculation temperatures as main units and cultivars or lines and fertilizer levels as subunits crossing each other. The first trial was made from January 14 to 28, and the second trial from January 29 to February 12, 1982.

Experiment III: Pre- and Post-inoculation Temperature Effects

The purpose of this experiment was to determine if pre- and post-inoculation temperatures and their interactions would affect responses of certain wheat lines resistant to WSM. Only resistant lines C.I. 15321, C.I. 15322, and OK 9387A were used, since the previous studies indicated blue Jacket and Wichita were highly susceptible to WSM at all temperatures tested. Twenty-one day old plants in pots were moved from greenhouse into growth chambers with temperature settings of 25, 30, 35 C with a 12 hr photoperiod. They were incubated at each respective temperature for 7 days before being inoculated with WSMV as in previous experiments. After inoculation, pots were moved into growth chambers in such arrangement so that each line in each post-inoculation temperature regime would have one pot of plants from 25 C, one from 30 C, and one from 35 C pre-inoculation temperature. The classification of response was the same as in previous experiments.

The experimental design was a split plot with post-inoculation temperatures as main units, and pre-inoculation temperatures and lines as subunits in a factorial arrangement. The experiment was done twice and each trial was treated as a block. The first trial was made from March 14 to 28, and the second from May 31 to June 13, 1982.

Since all three supposedly WSM resistant lines expressed systemic symptoms of WSM at the high temperature, it was important to know the fate of WSMV in these supposely resistant plants. An infectivity assay was conducted to determine if the resistant lines were symptomless carriers of WSMV at low temperature, or if the virus was eliminated or localized when plants were incubated in low temperature. Plants of the

three resistant lines incubated at 25 or 30 C before inoculation and then maintained at 25 C post-inoculation temperature without showing systemic symptoms of WSM were singly ground with motar and pestle together with a few drops of tap water and a small amount of Celite. Sap thus extracted was used to inoculat greenhouse grown, healthy Blue Jacket leaves manually. Inoculated leaves and non-inoculated newly grown leaves from experiment plants were separately assayed. Plants with systemic symptoms and dying plants without systemic symptoms held at high temperatures (30 or 35 C) also were assayed. Manually inoculated plants were kept at 25 C in a growth chamber with 12 hr photoperiod for 14 days. The evaluation was based on the appearance of systemic symptoms of WSM.

Experiment IV: Pre- and Post-inoculation Temperature and Photoperiod Effects

The effects of both pre- and post-inoculation temperatures and photoperiods and their interactions on response of wheat lines resistant to WSM were studied. Two temperature regimes, 25 and 30 C, and two photoperiod levels, 12 and 15 hr, were used for both pre- and post-inoculation treatments. The wheat lines, C.I. 15321, C.I. 15322, and OK 9387A, were grown in pots in the greenhouse for 21 days as described for experiment III before being moved into growth chambers with different temperature and photoperiod combinations. Seven days later, all plants were inoculated with WSMV. They then were placed in growth chambers with different temperature - photoperiod combinations so that each growth chamber would contain four pots of plants of each line, each pot being from a different pre-inoculation temperature -

photoperiod combination. Evaluation of response was accomplished as in the previous experiments.

The experimental design was a split plot with post-inoculation temperatures and photoperiods as main units in a factorial arrangement, and pre-inoculation temperatures and photoperiods as well as wheat lines as subunits also in a factorial arrangement. The experiment was made twice and each trial was treated as a block. The first trial was made from Feburary 21 to March 7, and the second from May 23 to June 6, 1982.

Experiment V: Genetics of Resistance in a Wheat cross, OK 9387A/Payne

The purpose of this experiment was to determine the segregation pattern of resistance to WSM in an F_2 population of OK 9387A/payne, and the effect of post-inoculation temperature on the segregation pattern. Each of four wooden flas was divided into ten rows approximately 3.3 cm apart. The first row was planted with 10 seeds of C.I. 15322 and OK 9387A as resistant checks. One hundred twenty-one seeds of the F_2 population were approximately equally distributed into the remaining nine rows. The flats were held in the greenhouse until the plants were 28 days old. The plants were inoculated with WSMV as previously described. Immediately after inoculation, two flats were placed individually in growth chambers with temperature settings at 25 and 30 C, respectively, with a 12 hr photoperiod. One flat was kept in the greenhouse and one was placed outdoors. Evaluation of the reponse of each plant was made 14 days after inoculation and was based on the development of systemic symptoms of WSM. Plants were classified as either resistant or susceptible. The experiment was made from April 8 to 22, 1982.

Data Analysis

Since the susceptibility was expressed as the percentage of total inoculated plants showing systemic symptoms of WSM, except for the infectivity assay and experiment V, the percentage figure was transformed by inverse sine ($\sin^{-1}$) transformation for unequal denominators as suggested by Steel and Torrie (48). Standard statistical analyses were performed based on transformed data; however, some data presented in the next chapter contain original, non-tansformed data.

CHAPTER IV

RESULTS

Experiment I: Post-inoculation Temperature Effects

At low post-inoculation temperatures (20 and 25 C), typical light green mottling systemic mosaic symptoms of WSM first appeared on newly emerging, non-inoculated leaves of the susceptible host Wichita and the tolerant host Blue Jacket. After the full expansion of those leaves, streaks of light green, and later yellow, appeared on the dark green background. Wichita had the most severe symptoms, with this cultivar leaves with streak mosaic symptoms were quite yellowish.

At 20 C, the resistant lines, C.I. 15321, C.I. 15322, and OK 9387A, did not produce systemic symptoms of WSM on non-inoculated newly emerged leaves, but mottling did appear around inoculation sites on inoculated leaves which later turned into yellow streaks. Wichita and Blue Jacket may or may not express this localized reaction of inoculated leaves at any of the temperature regimes tested. At 25 C, a few plants of the resistant lines developed systemic symptoms along with the localized reaction. At 30 C, more plants of the resistant genotypes developed systemic symptoms and some plants with systemic symptoms died before termination of the experiment. At 35 C, almost all plants died before or immediately after the response evaluation began. Only a few plants expressed mosaic symptoms. Plants which survived for 14 days after

inoculation were weak and had tip necrosis and/or chlorosis compared to the check plants which appeared healthy although somewhat retarded in growth. Apparently, the expression of WSM symptoms was inhibited at 35 C. The dead or dying plants did not show root rot symptoms or any other disorders, and later infectivity assay revealed a systemic distribution of WSMV in those plants which could still be assayed. Therefore dead or dying plants without systemic symptoms of WSM were classified as if they had actually had systemic symptoms. Figure 1 shows the localized reaction on inoculated leaves of resistant lines at 20 C. Figure 2 shows the systemic symptoms on seedlings of resistant lines which developed at 25 C post-inoculation temperature.

The progression of systemic symptom development and final percentage of plants with systemic symptoms on the five cultivars and lines tested are shown graphically in Figure 3-7. At 20 and 25 C, resistant lines either developed no systemic symptoms, or only a small percentage of plants developed symptoms. Also the incubation period was long and the rate of symptom development was slow. For Wichita and Blue Jacket, the rate of systemic symptom development was slower and the final percentage of plants with systemic symptoms were less at 20 C than at 25 C. At 30 and 35 C, not much difference occurred between these susceptible cultivars and the resistant lines in terms of rate of disease development and the final percentages of plants with systemic symptoms.

Statistical analysis of the data indicated that 14 days after inoculation, there were significant differences between post-inoculation temperatures and between cultivars and lines (Table 1). There was no significant difference between blocks (trials), but there was a significant interaction between cultivars and lines and post-inoculation

Figure 1. Localized Streak Mosaic Symptoms of WSM on Inoculated Leaves of Supposedly Resistant Wheat Lines. Plants Were Maintained at a Post-inoculation Temperature of 20 C.

Figure 2. Systemic Symptoms of WSM on Newly Emerging Leaves of Supposedly Resistant Wheat Lines. Plants Were Maintained at a Post-inoculation Temperature of 25 C.

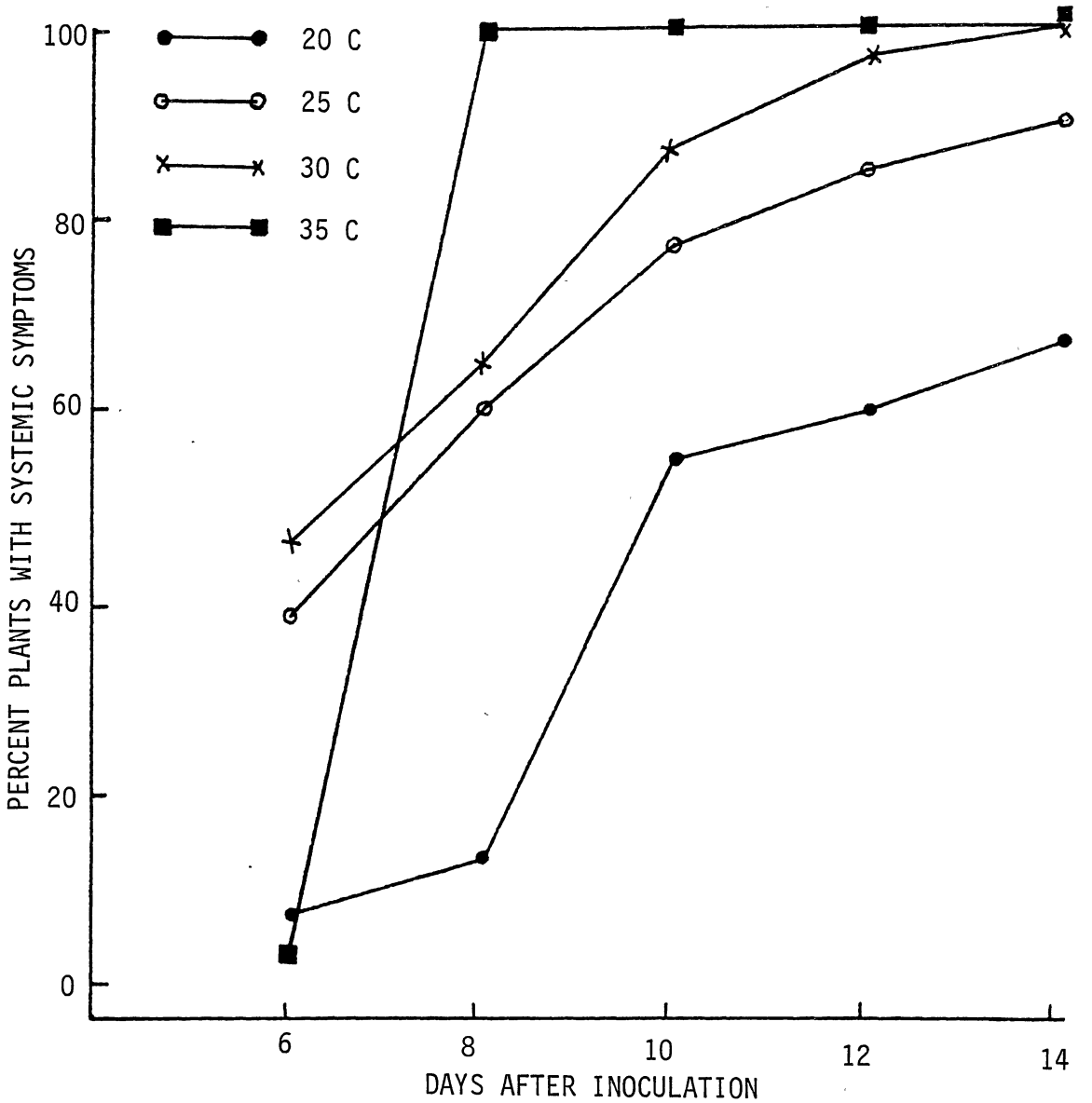

Figure 3. Progressive Development of Systemic Symptoms of WSM on the Wheat Cultivar Wichita at Four Different Post-inoculation Temperatures

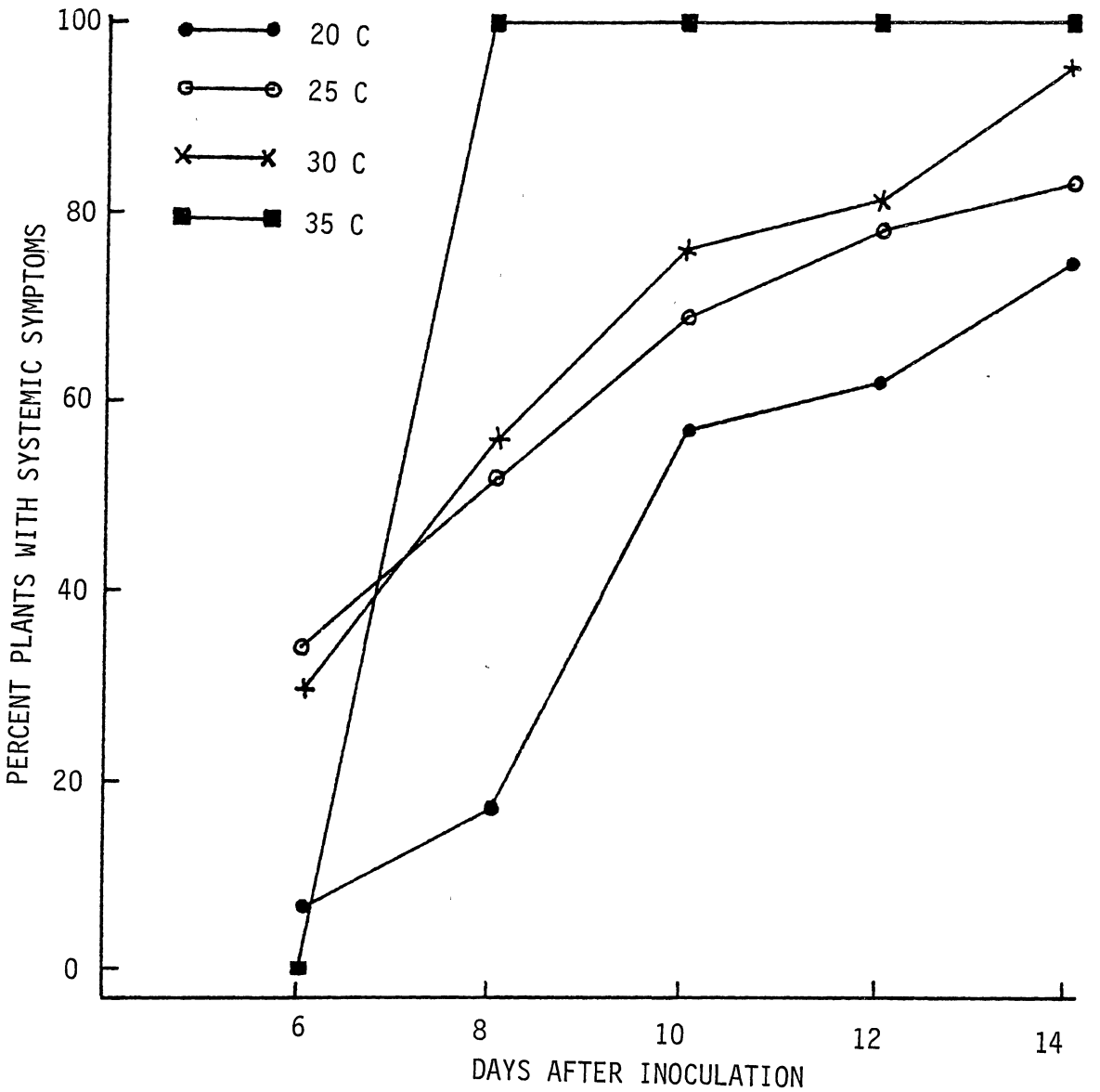

Figure 4. Progressive Development of Systemic Symptoms of WSM on the Wheat Cultivar Blue Jacket at Four Different Post-inoculation Temperatures

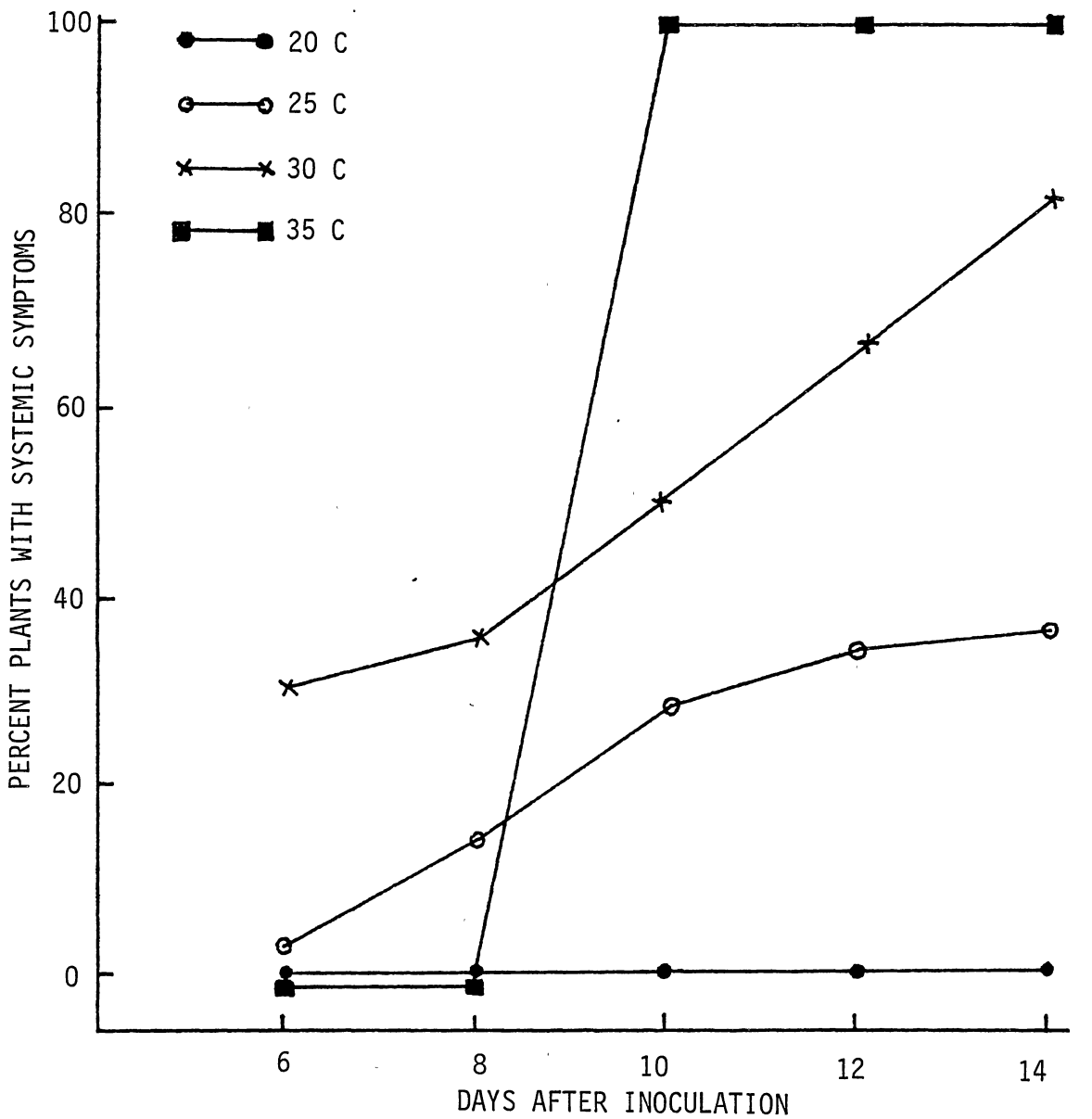

Figure 5. Progressive Development of Systemic Symptoms of WSM on Wheat Line C.I. 15321 at Four Different Post-inoculation Temperatures

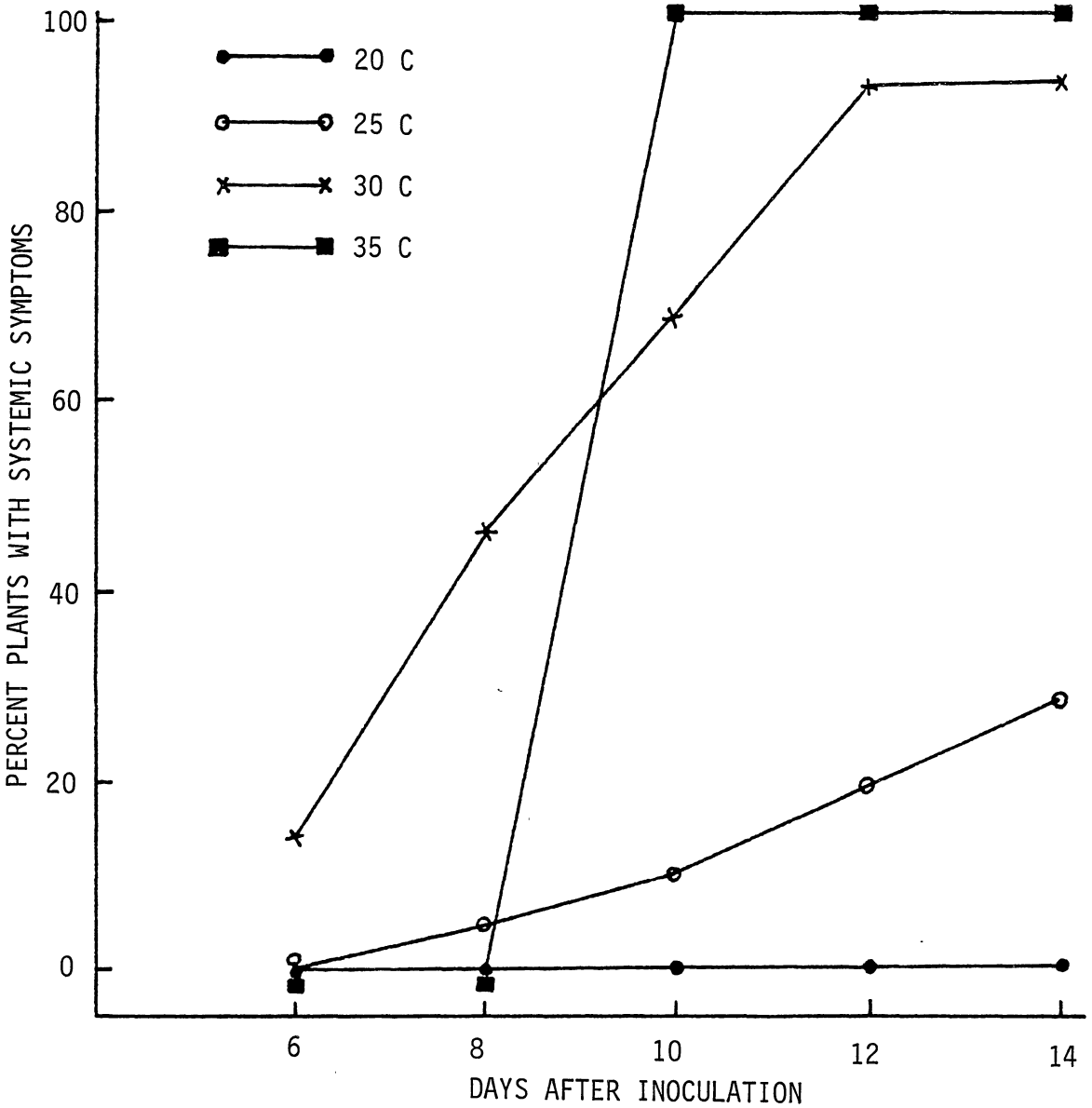

Figure 6. Progressive Development of Systemic Symptoms of WSM on Wheat Line C.I. 15322 at Four Different Post-inoculation Temperature

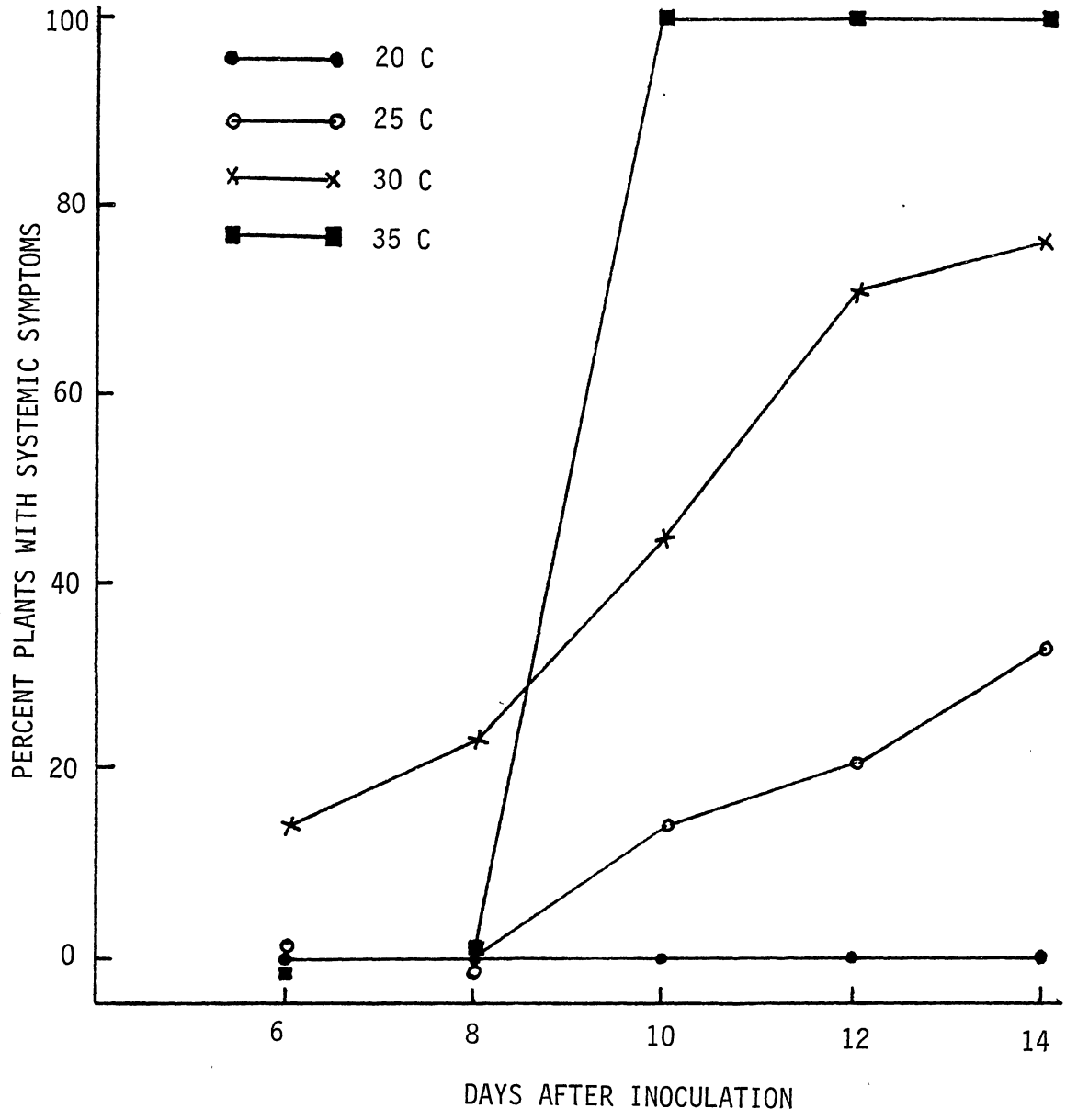

Figure 7. Progressive Development of Systemic Symptoms of WSM on Wheat Line OK 9387A at Four Different Post-inoculation Temperature

TABLE I

ANALYSIS OF VARIANCE OF POST-INOCULATION TEMPERATURE EFFECTS ON THE RESPONSE OF CERTAIN WHEAT CULTIVARS AND LINES TO WSMV INFECTION 1/

Source of Variation	df	Mean Square	F Value
Block	2	2929.87	4.11 ns 2/
Temperature	3	16896.55	23.71 ** 3/
Error(a)	6	712.71	
Genotype	4	4551.28	20.48 **
Temperature x Genotype	12	1038.50	4.67 **
Error(b)	32	222.19	8.67 **
Sample Error	60	25.36	8.67

1/ Analysis of variance was performed on transformed data ($\sin^{-1}\sqrt{\text{percentage}}$) collected 14 days after inoculation.

2/ ns = Not significant at 0.05 level.

3/ ** = Significant at 0.01 level.

temperature. This was undoubtedly due to the differences between the resistant wheat lines and the susceptible wheat cultivars at the low temperatures. Using Duncan's multiple range test the cultivars and lines fell into two distinct groups; those which were classified as susceptible, and those classified as resistant (Table II).

Although differences between blocks were not statistically significant, there was a noticeable difference between certain blocks, or trials, particularly with the supposedly WSM resistant lines (Figure 8). Since the trials were made over a period of times, from May to the end of July, pre-inoculation temperature and photoperiod in the greenhouse could have been factors predisposing these plants to WSMV infection. Also the amount of fertilizer supplied could have been a factor since fertility levels in this experiment were not quantitatively controlled. Therefore, further experiments were designed to study the effects of pre-inoculation temperatures and fertility levels as well as photoperiods on systemic symptom expression of WSM.

Experiment II: Fertilizer and Post-inoculation Temperature Effects

Significant differences were indicated between the cultivars and lines but none were found between fertilizer levels, post-inoculation temperatures, nor in the interaction of these two factors (Table III).

The amount of fertilizer applied after inoculation did not increase the number of plants of the resistant lines exhibiting systemic symptoms of WSM. Also, with high levels of fertilizer and low post-inoculation temperatures, plants of all five cultivars and lines tested had retarded growth, dark green leaf color, and tended to wilt even with ample supplies of water. Plants which received the lowest level

TABLE II

COMPARISON OF THE RESPONSE OF CERTAIN WHEAT CULTIVARS AND LINES TO WSMV INFECTION 1/

Cultivar or Line	Percent of Plants with Systemic Symptoms	
	Experiment I 2/	Experiment II 3/
Wichita	89.17 a 4/	96.53 a
Blue Jacket	87.92 a	91.94 a
C.I. 15321	53.38 b	7.11 b
C.I. 15322	55.13 b	10.43 b
OK 9387A	52.17 b	8.61 b

1/ Data collected 14 days after inoculation.

2/ Mean of 4 temperatures, each with 6 samples.

3/ Mean of 3 temperatures, each with 6 samples.

4/ Numbers in the same column with the same letter are not different at 0.05 level by Duncan's Multiple Range Test.

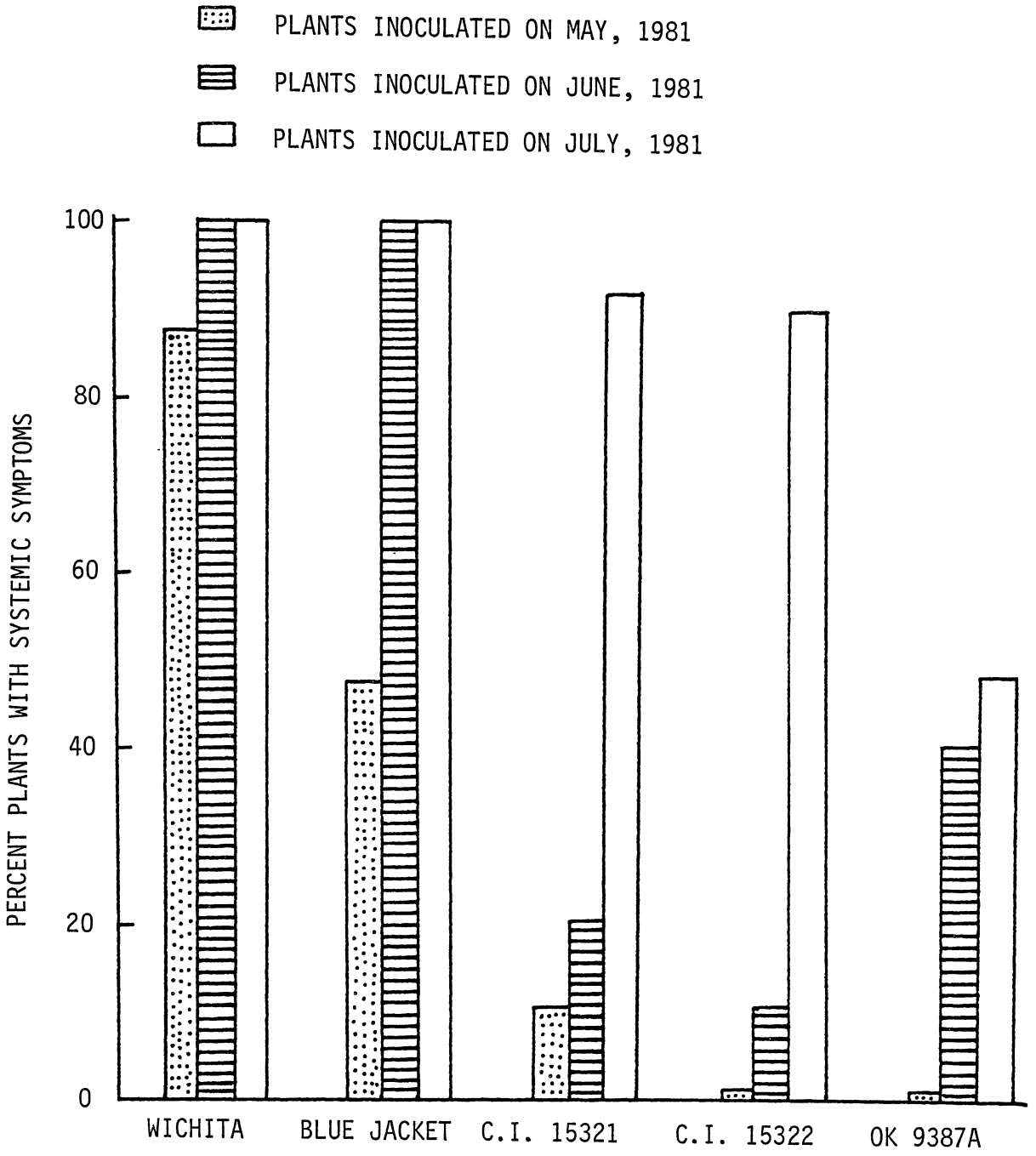

Figure 8. Differences in Percent of Plants with Systemic Symptoms of Five Wheat Cultivars and Lines Inoculated with WSMV at Different Times of the Year. Plants Were Maintained at 25 C Post-inoculation Temperature

TABLE III

ANALYSIS OF VARIANCE OF THE EFFECTS OF POST-INOCULATION TEMPERATURES AND OF FERTILIZER LEVELS ON THE RESPONSE OF CERTAIN WHEAT CULTIVARS AND LINES TO WSMV INFECTION 1/

Source of Variation	df	Mean Square	F Value
Block	1	55.28	0.13 ns 2/
Temperature (Temp)	2	5122.29	6.07 ns
Error(a)	2	421.70	
Genotype(Gen)	4	20457.43	159.85 ** 3/
Temp x Gen	8	240.78	1.88 ns
Error(b)	12	127.98	
Fertilizer(Fert)	2	1.58	0.21 ns
Temp x Fert	4	22.05	2.99 ns
Error(c)	6	7.37	
Gen x Fert	8	13.20	1.53 ns
Temp x Gen x Fert	16	16.21	1.88 ns
Error(d)	24	8.61	

1/ Analysis of variance was performed on transformed data ($\sin^{-1}\sqrt{\text{percentage}}$) collected 14 days after inoculation.

2/ ns = Not significant.

3/ ** = Significant at 0.01 level.

of fertilizer (1 gm per pot) had rapid growth, natural color, and did not show water deficiency symptoms.

Although not statistically significant, the appearance of some plants with systemic symptoms in the resistant lines at a 30 C post-inoculation temperature is similar to the results of experiment I (Table IV). The low percentage of plants expressing systemic symptoms in the resistant lines in this experiment at 25 and 30 C may again be due to a predisposition effect of pre-inoculation temperature and photo-period, since this experiment was made during January and Febuary, 1982, when ambient greenhouse temperatures would have been lower and photo-period shorter than in the May to July period.

Experiment III: Pre- and Post-inoculation Temperature Effects

The three WSM resistant lines were essentially the same in their response to both pre- and post-inoculation temperatures (Figure 9-11). Pre-inoculation temperature effects were similar to the effects of post-inoculation temperature determined in the experiment I and repeated here. The higher the temperature the greater the number of plants with systemic symptoms in both cases. Possibly due to the experimental design, the pre-inoculation temperature effects were significantly different but the post-inoculation temperature effects were not (Table V). The additive effect of pre- and post-inoculation temperatures was to further increase the number of plants with systemic symptoms. Correlation coefficients between the sum of pre- and post-inoculation temperatures and the percentage of plants with systemic symptoms exposed to those temperatures were significant at 0.05 level for all three resistant lines (Table VI).

TABLE IV

EFFECTS OF POST-INOCULATION TEMPERATURE ON THE APPEARANCE OF SYSTEMIC SYMPTOMS OF WSM ON CERTAIN WHEAT CULTIVARS AND LINES

Cultivar or Line	Percent of Plants with Systemic Symptoms Post-inoculation Temperature		
	20 C	25 C	30 C
Wichita	93 1/ a 2/	97 a	100 a
Blue Jacket	77 a	100 a	99 a
C.I. 15321	0 b	0 b	21 b
C.I. 15322	0 b	0 b	30 b
OK 9387A	0 b	0 b	26 b

1/ Each figure is an average of three fertilizer levels and two blocks or trials, collected 14 days after inoculation.

2/ Cultivars or lines with the same letter are not different at 0.05 level by Duncan's Multiple Range Test.

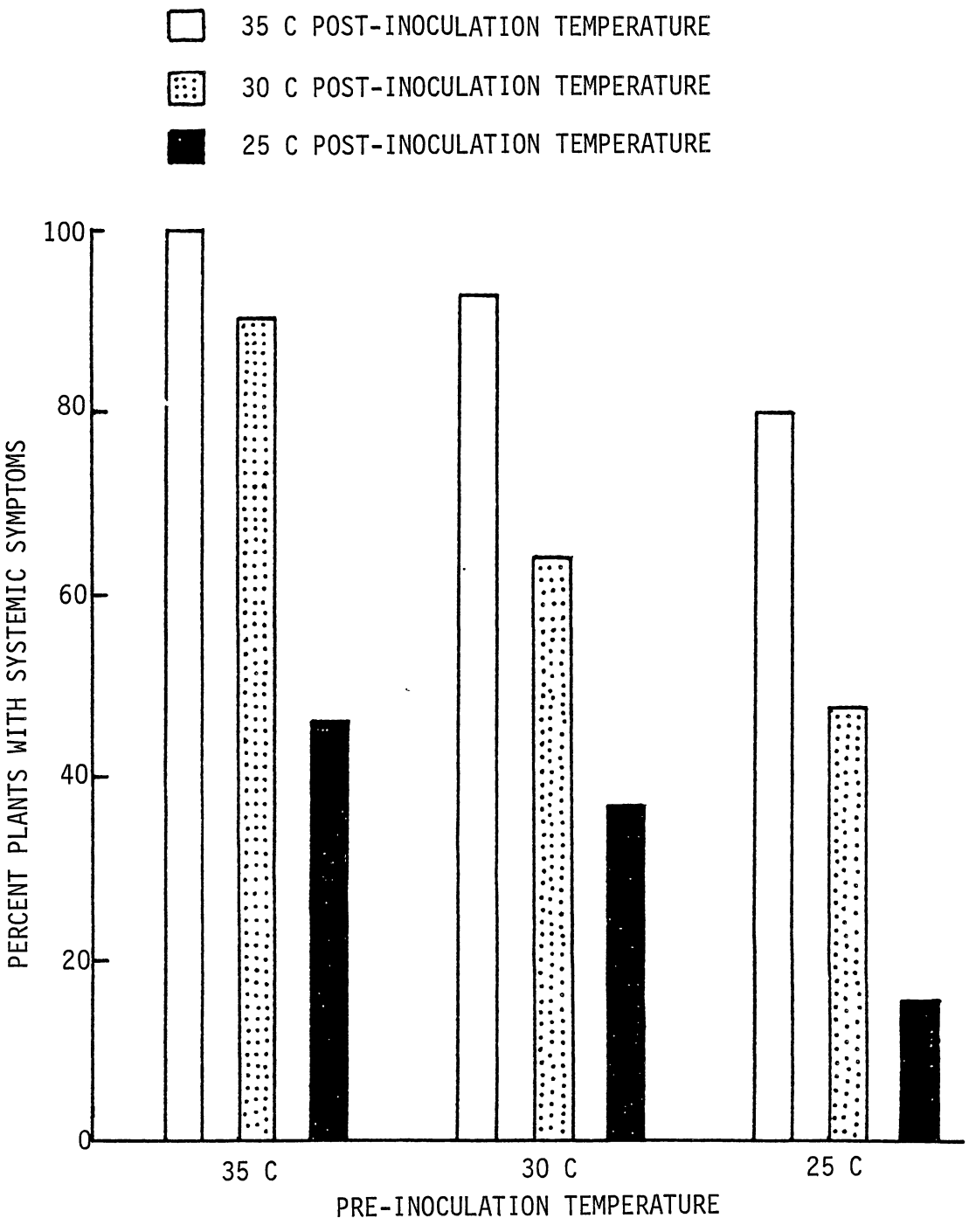

Figure 9. Cumulative Effect of Pre- and Post-inoculation Temperatures on the Percent of Plants with Systemic WSM Symptoms in the Wheat Line C.I. 15321

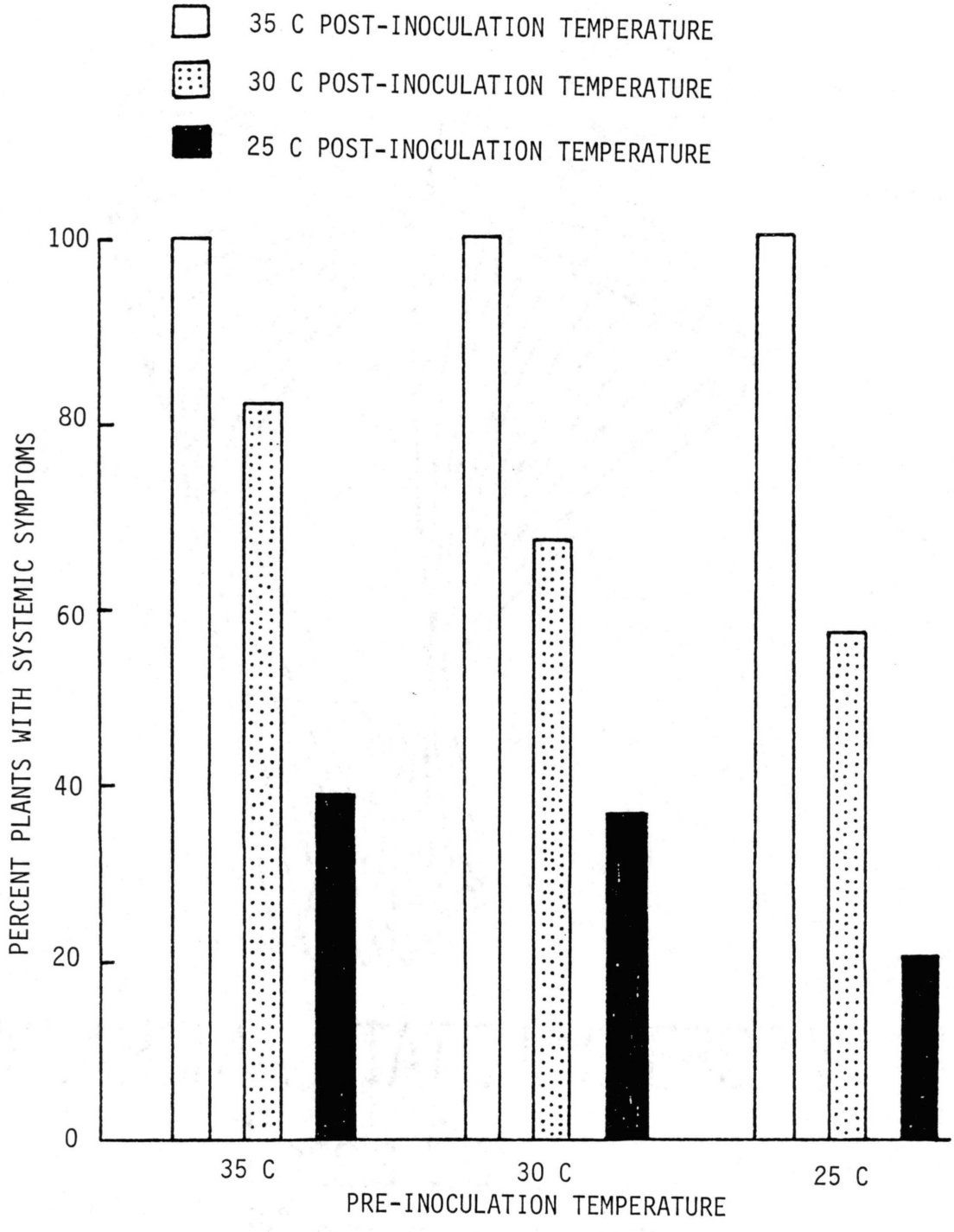

Figure 10. Cumulative Effect of Pre- and Post-inoculation Temperatures on the Percent of Plants with Systemic WSM Symptoms in the Wheat Line C.I. 15322

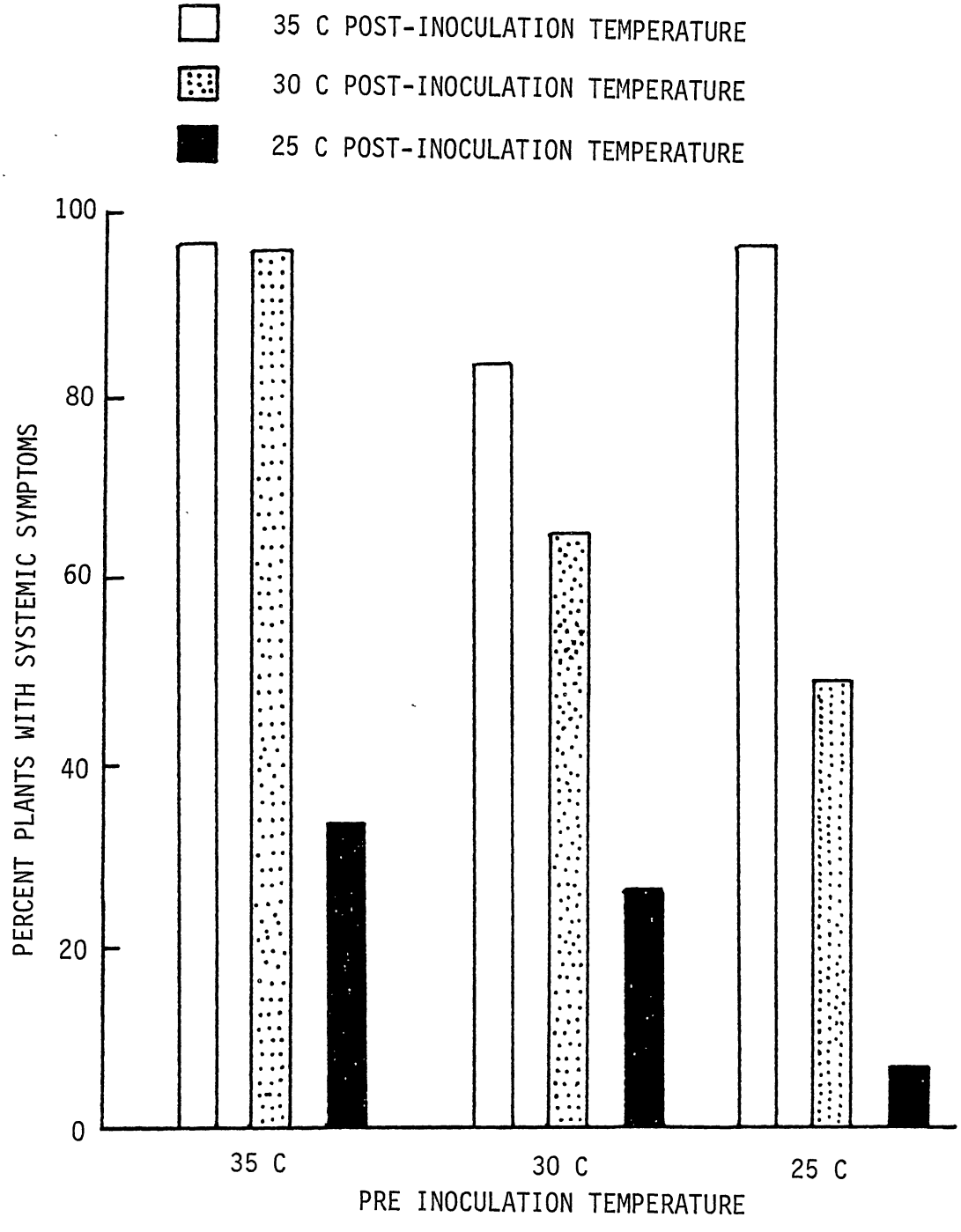

Figure 11. Cumulative Effect of Pre- and Post-inoculation Temperatures on the Percent of Plants with Systemic WSM Symptoms in the Wheat Line OK 9387A

TABLE V

ANALYSIS OF VARIANCE OF THE PRE- AND POST-INOCULATION TEMPERATURE EFFECTS ON THE PERCENT OF PLANTS WITH SYSTEMIC WSM SYMPTOMS ON THREE RESISTANT WHEAT LINES 1/

Source of Variation	df	Mean Square	F Value
Block	1	5328.63	3.38 ns 2/
Post-inoculation Temperature (PItemp)	2	10244.04	6.49 ns
Error(a)	2	1577.82	
Pre-inoculation Temperature (PDtemp)	2	1163.88	9.42 ** 3/
Genotype	2	56.37	0.46 ns
PDtemp x Genotype	4	86.29	0.70 ns
PItemp x PDtemp	4	186.18	1.51 ns
PItemp x Genotype	4	77.98	0.58 ns
PItemp x PDtemp x Genotype	8	25.67	0.21 ns
Error(b)	24	123.56	

1/ Analysis of variance was performed on transformed data ($\sin^{-1}\sqrt{\text{percentage}}$) collected 14 days after inoculation.

2/ ns = Not significant.

3/ ** = Significant at 0.01 level.

TABLE VI

CORRELATION BETWEEN THE SUM OF PRE- AND POST-INOCULATION TEMPERATURES AND THE PERCENT OF PLANTS OF THREE RESISTANT LINES OF WHEAT WITH SYSTEMIC WSM SYMPTOMS

Wheat line	Correlation Coefficient 1/
C.I. 15321	0.732 * 2/
C.I. 15322	0.635 *
OK 9387A	0.748 *

1/ Calculation was based on transformed data ($\sin^{-1}\sqrt{\text{percentage}}$) collected 14 days after inoculation.

2/ * = Significant at 0.05 level with 8 degree of freedom.

The infectivity assay was made only to identify possible symptomless carriers, and no attempt was made to measure the concentration of WSMV in the assayed plants. The test indicated that plants of resistant lines held at 25 C post-inoculation temperature and without systemic symptoms of WSM did not contain WSMV, or if they did, not in sufficient quantity in the non-inoculated newly emerging leaves to be infective. (Table VII). However, inoculated leaves from the same plants sometimes contained sufficient WSMV to be infective. The number of plants assayed with inoculated leaves expressing WSM symptoms was so low in all three resistant lines that WSMV probably existed in such a very low concentration that it was not easily recovered. On the other hand, plants of resistant lines that developed systemic symptoms, regardless of the temperature treatment they received, always contained infective level of WSMV. Dead plants classified in the group with systemic symptoms had systemically distributed transmissible WSMV and it seems probable that death was caused by WSMV infection.

Experiment IV: Pre- and Post-inoculation Temperature and Photoperiod Effects

Although the percent of plants which developed systemic symptoms with different photoperiods was not statistically significant (Table VIII), when the post-inoculation temperature was high the percent of plants with systemic symptoms was highest in almost all cases with longer photoperiod (Table IX). This was not true, however, when post-inoculation temperature was low.

With this experimental design both pre- and post-inoculation temperatures were significantly different in the number of plants

TABLE VII

INFECTIVITY ASSAY OF WSMV-INOCULATED PLANTS
OF THREE WSM RESISTANT WHEAT LINES
FROM EXPERIMENT III

PDtemp 1/	PItemp 2/		Wheat Line C.I. 15321	C.I. 15322	OK 9387A
Without Systemic Symptoms					
25 C	20 C	New Leaf	0/12 3/	0/13	0/14
30 C	25 C	New leaf	0/13	0/11	0/12
25 C	25 C	Inoculated Leaf	1/11	3/11	1/11
With Systemic Symptoms					
Plant Alive, New Leaf			6/6	7/7	7/7
Plant Dead			4/4	4/4	3/3

1/ PDtemp = Pre-inoculation temperature.

2/ PItemp = Post-inoculation temperature.

3/ Numerator: Number of assayed plants that induced systemic symptoms in the cultivar Blue Jacket.

Denominator: Total number of plants assayed.

TABLE VIII

ANALYSIS OF VARIANCE OF PRE- AND POST-INOCULATION TEMPERATURE AND PHOTOPERIOD EFFECTS ON THE PERCENT OF PLANTS WITH SYSTEMIC WSM SYMPTOMS IN THREE RESISTANT WHEAT LINES 1/

Source of Variation 2/	df	Mean Square	F Value
Block	1	359.56	2.41 ns 3/
Post-inoculation Temperature (PItemp)	1	69138.23	463.14 ** 4/
Post-inoculation Photoperiod (PIperiod)	1	1053.30	7.05 ns
PItemp x PIperiod	1	570.61	3.82 ns
Error(a)	3	149.27	
Pre-inoculation Temperature (PDtemp)	1	1160.44	14.00 **
Pre-inoculation Photoperiod (PDperiod)	1	9.95	0.12 ns
Genotype	2	374.61	4.52 * 5/
PItemp x Genotype	2	379.82	4.58 *
Error(b)	43	82.88	

1/ Analysis of variance was performed on transformed data ($\sin^{-1}\sqrt{\text{percentage}}$) collected 14 days after inoculation.

2/ Other sources of variation not listed were not significant at 0.05 level.

3/ ns = Not significant.

4/ ** = Significant at 0.01 level.

5/ * = Significant at 0.05 level.

TABLE IX

EFFECT OF PRE- AND POST-INOCULATION TEMPERATURES AND PHOTOPERIODS ON THE PERCENT OF PLANTS WITH SYSTEMIC WSM SYMPTOMS IN THREE RESISTANT WHEAT LINES

Temperature		Photoperiod		Wheat Line		
PD 1/	PI 2/	PD	PI	C.I. 15321	C.I. 15322	OK 9387A
30 C	30 C	15 hr	15 hr	100 % 3/	92 % 3/	100 % 3/
30	30	15	12	89	83	90
30	25	15	15	5	25	7
30	25	15	12	7	0	24
30	30	12	15	100	79	100
30	30	12	12	87	64	91
30	25	12	15	22	19	0
30	25	12	12	22	7	24
25	30	15	15	89	88	97
25	30	15	12	88	73	68
25	25	15	15	7	7	0
25	25	15	12	0	4	0
25	30	12	15	93	82	97
25	30	12	12	66	69	79
25	25	12	15	12	4	0
25	25	12	12	0	9	0
Mean at 30 C Post-inoculation Temperature				89	79	90
Mean at 25 C Post-inoculation Temperature				9	9	7
Mean at 30 C Pre-inoculation Temperature				54	46	54
Mean at 25 C Pre-inoculation Temperature				44	42	43
Overall Mean				49	44	49

1/ PD = Pre-inoculation.

2/ PI = Post-inoculation.

3/ Percent of plants with systemic WSM symptoms.

showing systemic symptoms and in this experiment there was a difference between wheat lines and in the wheat lines by post-inoculation temperature interaction at 0.05 level. C.I. 15322 had slightly fewer plants with systemic symptoms than the other two lines, but this was true only at high post-inoculation temperature. The lines were not different at the low post-inoculation temperature.

Experiment V: Genetics of Resistance in a Wheat Cross, OK 9387A/Payne

The phenotypic expression of WSM resistance in an F_2 population of OK 9387A/Payne was much affected by the post-inoculation temperature to which the population was subjected (Table X). At 30 C in a growth chamber, 109 of 114 F_2 plants had systemic symptoms of WSM or died before the evaluation began. The five plants without systemic symptoms were weak and developed systemic symptoms of WSM later. The F_2 plants held at a post-inoculation temperature of 25 C in a growth chamber had 86 plants with systemic symptoms out of total of 112 plants. In the greenhouse at a temperature of 23 $\pm$ 10 C, 96 out of 116 F_2 plants had systemic symptoms. Of those kept outdoors at 17 $\pm$ 7 C only 72 out of 110 F_2 plants had systemic symptoms.

If it were assumed that one recessive gene in OK 9387A conditioned resistance, then the F_2 population maintained in a growth chamber at 25 C post-inoculation temperature would fit this model very well ($0.5 < P < 0.75$), as would the F_2 population in the greenhouse ($0.05 < P < 0.1$). The F_2 population grown outdoors would be marginal ($0.01 < P < 0.025$), and the F_2 population maintained in a growth chamber at 30 C post-inoculation temperature would not fit this model at all (Table X). A

TABLE X

REACTION OF F_2 POPULATIONS OF THE CROSS OK 9387A/PAYNE TO WSM UNDER DIFFERENT POST-INOCULATION TEMPERATURES 1/

	Number of Plants				
	Total	Symptomless	Systemic Symptoms	X^2 2/	P 3/
30 C Growth Chamber					
C.I. 15322	5	3	2		
OK 9387A	10	7	3		
F_2	114	5	109	25.84	$<.01$
25 C Growth Chamber					
C.I. 15322	9	7	2		
OK 9387A	9	7	2		
F_2	112	26	86	0.19	$.5<P<.75$
Greenhouse					
C.I. 15322	7	6	1		
OK 9387A	10	8	2		
F_2	116	20	96	3.72	$.05<P<.1$
Outdoors					
C.I. 15322	8	8	0		
OK 9387A	10	10	0		
F_2	110	38	72	5.35	$.01<P<.025$

1/ Homogeneity test value (32.84) has the probability of goodness of fit of less than 0.005, based on 3 degree of freedom.

2/ X^2 = Chi square value.

3/ P = Probability of fitting single recessive resistance gene model, based on 1 degree of freedom.

homogeneity test indicated that these four populations were heterogeneous, which was interpreted to indicate that post-inoculation temperature had a definite effect on the phenotypic expression of resistance of the F_2 plants tested.

Plants of the check lines, C.I. 15322 and OK 9387A, were similarly affected by temperature in this experiment. It is worth mentioning here that plants of these lines held outdoors did not develop any systemic symptoms of WSM, whereas some plants of both lines did develop systemic symptoms in the other three locations.

CHAPTER V

DISCUSSION

The effects of post-inoculation temperature on the response of host plants to virus infection has been demonstrated in various host-virus systems. In this study, it was demonstrated that there was a distinct difference in the percentage of inoculated plants which developed systemic WSM symptoms in the wheat lines C.I. 15321, C.I. 15322, and OK 9387A among different post-inoculation temperature regimes. Pfannenstiel and Niblett (27) reported that neither C.I. 15321 nor C.I. 15322 exhibited complete resistance to WSM even at 18 C post-inoculation temperature. This study did not substantiate that report since complete resistance was exhibited by these lines at 20 C. In this respect this study corresponds more closely with that reported by Martin (19) who showed that C.I. 15322 did not develop systemic symptoms of WSM at 22 C.

Varying the level of nutrition did not indicate any effect on the number of plants developing systemic symptoms of WSM of either the resistant or susceptible wheat cultivars or lines. The difference between this study and others could have resulted from the timing of fertilizer application. In several reports (9, 14, 51), nutrients were supplied prior to inoculation and thereafter, instead of after the inoculation as in this study. The later application, as shown in this study, did affect plant growth, however, since the high levels of

fertility caused significant retardation of growth before the plants were evaluated for disease response.

Predisposing plants for a period of seven days to high temperature significantly changed the response of resistant wheat lines to subsequent development of WSMV in the plants. Although the predisposition effect of temperature on host susceptibility has been reported (13, 18, 26, 52), most of those experiments were made with dicotyledous plants tested with viruses which produced a local lesion reponse. In the present study, high pre-inoculation temperature was shown to change a resistant host to susceptible one in the wheat - WSMV system. This study demonstrated that growing plants in a greenhouse condition, as was done in some experiments in this study, could result in misleading data and conflicting conclusions.

The effects of photoperiod and temperature on plants are difficult to separate. Meiners (24), for example, reported that in experiments in the growth chamber, five snap bean genotypes were resistant to peanut stunt virus infection at 30 C day and 23 night temperatures with a 16 hr photoperiod, but were susceptible at 25 C day and 18 C night temperatures with a 10 hr photoperiod. Unfortunately, there was no factorial arrangement of temperature - photoperiod combinations. Thus the effect of photoperiod can not be separated from that of temperature. In this study, an attempt was made to separate the effects of both pre-inoculation and post-inoculation temperatures from photoperiod effects. Although photoperiod has been shown to affect virus multiplication in a susceptible host (6, 31), nothing has been reported on the influence of photoperiod on host normally considered resistant to virus infection. In this study it was shown that, at least in the

wheat - WSMV combination, photoperiod is not a factor affecting the expression of resistance of the three resistant lines tested.

Since systemic symptoms of WSM which developed on uninoculated newly emerging leaves of the three resistant wheat lines used in this study were often very faint - many times being only single light green streak at the end of 14 days after inoculation, the question arises whether the virus content of those leaves in such circumtance is sufficient to be transmissible. Chantarasnit (5) reported that even symptomless newly emerging leaves of the wheat lines C.I. 15321 and C.I. 15322 contained a transmissible quantity of WSMV. However, she pooled all leaves and all plants together to obtain the plant sap used for her infectivity assay, including the inoculated leaves. In this study, the infectivity assay was made on an individual plant and individual leaf basis, which should give a more accurate measure of the plants and leaves which contained transmissible virus. Newly emerging leaves with systemic symptoms did contain transmissible virus and indicated that in these cases resistance of these lines had been altered by the environmental conditions encountered. The lack of infectivity in non-inoculated leaves of plants without systemic symptoms indicated that WSMV in these resistant lines was not translocated to other parts of plants; hence they were not symptomless carriers. Similar results have been reported also by Hendrix (15) with the tobacco - tobacco ring spot virus combination. In this experiment, the inoculated leaves often retained sufficient virus to be transmissible, regardless of whether other leaves on the plant had systemic symptoms or not.

Most virus disease resistance transferred to cultivated crops from wild relatives is conditioned by a single gene. Tobacco mosaic

resistance in tobacco transferred from N. glutinosa is conditioned by a single dominant gene (21). Resistance to cucumber mosaic virus 1 in spinach obtained from a wild Asian collection of Spinacia oleracea is conditioned by single dominant gene (30). In this study, it was found that with the proper post-inoculation temperature, an F_2 population of the cross OK 9387A/Payne fitted the ratio expected for a single recessive gene conditioning resistance. However, Sebesta (37) found that segregation in F_2 from a cross involving OK 9387A did not fit a single gene model. The contradictory results probably were caused by the temperature effect. His study was made in a greenhouse during October, 1980, a time when the greenhouse temperature could have exceeded the maximum temperature which would permit the free expression of resistance.

Temperature effects on expression of virus disease symptoms can be influenced by genetic background. The F_2 progenies of crosses of the tomato breeding line 801, for instance, segregated into two definite patterns at low and high temperatures, but both patterns conferred a single gene conditioning resistance (7). Crosses of line 825, which derived its resistance to tobacco mosaic virus from line 801, segregated without a definitive pattern at high temperature (28). In the present study, both high and low temperatures (30 C in a growth chamber and the ambient relatively low temperature of April, 1982) alter the resistant : susceptible ratio in an F_2 population of OK 9387A/Payne to WSM. But phenotypic segregation fitted single recessive resistant gene model when F_2 plants were incubated at 25 C in growth chamber or in greenhouse. The genetic background of susceptible parent of the F_2 population seems no effect on segregation.

Throughout this study no attempt was made to classify severity of systemic symptoms. Distinct differences in the degree of severity of symptoms were observed between Wichita and Blue Jacket, however, and although early systemic symptoms of the plants of the resistant lines were often faint, ultimately the symptoms became just severe as those on wichita.

CHAPTER VI

SUMMARY

1. Two hard red winter wheat cultivars, Wichita and Blue Jacket, two Agrotricum derivatives, C.I. 15321 and C.I. 15322, and a derived line from C.I. 15322, OK 9387A, were used to evaluate effects of post-inoculation temperature on WSM symptom expression. The number of plants showing systemic WSM symptoms 14 days after inoculation increased steadily at temperatures from 20 C through 35 C. The supposedly WSM resistant Agrotricum derivatives had no symptoms at 20 C, but above that temperature all lines had increasing numbers of plants with systemic symptoms with increasing temperature.
2. Levels of fertilizer applied immediately after inoculation did not affect the number of plants of the WSM resistant lines that expressed systemic symptoms. Pre- and post-inoculation photoperiods also did not have effect on resistance of Agrotricum derivatives to WSM.
3. Resistance of the Agrotricum derivatives to WSM was inversely related to pre-inoculation as well as post-inoculation temperatures. Newly emerged leaves of those plants which had WSM systemic symptoms were shown to contain transmissible virus by bioassay. Symptomless leaves did not contain transmissible virus, but many inoculated leaves still contained transmissible virus 14 days after inoculation. Inoculated plants without Systemic WSM symptoms on emerged

new leaves were not symptomless carriers of WSMV.

4. An F_2 population of OK 9387A/Payne produced different resistant : susceptible ratios when samples of that population were held at different post-inoculation temperatures. The F_2 samples held in a growth chamber at 25 C post-inoculation temperature or in a a greenhouse where the average ambient temperature was at 23 C, the segregation ratio indicated that resistance was conditioned by a single recessive gene ($P > 0.05$). At a post-inoculation temperature of 30 C in growth chamber, however, almost all F_2 plants tested developed systemic WSM symptoms and did not fit a single recessive gene model. Among F_2 plants held outdoors during April, 1982, at an ambient temperature averaging below 20 C, the ratio of resistant : susceptible plants fitted single recessive gene model, but only with P value less than 0.05.

LITERATURE CITED

1. Bawden, F. C. and B. Kassanis. 1950. Some effects of host nutrition on the susceptibility of plants to infection by certain viruses. Ann. Appl. Biol. 37: 46-57.

2. Brandes, J. and R. Bercks. 1965. Gross morphology and serology as a basis for classification of elongated plant viruses. Adv. in Virus Res. 11: 1-24.

3. Bremer, K. 1973. Resistance of native wheat (Triticum aestivum L.) populations collected in Turkey to the wheat streak mosaic virus. Phytopathologia Mediterranea 12: 116-117.

4. Carroll, T. W. and T. Kosuge. 1966. Effect of temperature on the response of systemically susceptible and hypersensitive Xanthi tobacco plants to tobacco mosaic virus infection. Phytopathology 56: 873 (abstract).

5. Chantarasnit, A. 1976. Comparative studies on the reaction of some winter wheats to wheat streak mosaic virus. Ph.D. Dissertation, Oklahoma State University.

6. Cheo, P. and G. S. Pound. 1952. Relation of air temperature, soil temperature, photoperiod, and light intensity to the concentration of cucumber virus 1 in spinach. Phytopathology 42: 306-310.

7. Cirulli, M. and L. J. Alexander. 1969. Influence of temperature and strain of tobacco mosaic virus on resistance in a tomato breeding line derived from Lycopersicon peruvianum. Phytopathology 59: 1287-1297.

8. Diachum, S. 1967. Effect of temperature on development of local lesions on tobacco leaves inoculated with tobacco streak virus. Phytopathology 57: 809 (abstract).

9. Foster, R. E. 1967. Chenopodium amaranticolor nutrition affects cucumber mosaic virus infection. Phytopathology 57: 838-840.

10. Foster, R. E. and R. E. Webb. 1966. High temperature masking of mosaic symptoms on muskmelon. Phytopathology 56 : 146 (abstract).

11. Gill, C. C. and P. H. Westdal. 1966. Effect of temperature on symptom expression of barley infected with aster yellow or

barley yellow dwarf viruses. Phytopathology 56: 369-370.

12. Gold, A. H., B. R. Houston, and J. W. Oswald. 1953. Electon-microscopy of elongated particles associated with wheat streak mosaic. Phytopathology 43: 458-459.

13. Ganzalaz, L. C. and G. S. Pound. 1963. The response to temperature of cabbage virus A infection in Nicotiana glutinosa. Phytopathology 53: 1041-1045.

14. Helms, K. and G. S. Pound. 1955. Host nutrition in relation to concentration of potato virus X and tobacco ring spot virus. Phytopathology 45: 567-573.

15. Hendrix, J. W. 1972. Temperature-dependent resistance to tobacco ringspot virus in L8, a necrosis-prone tobacco cultivar. Phytopathology 62: 1376-1381.

16. Johnson, J. 1921. The relation of air temperature to certain plant diseases. Phytopathology 11: 446-458.

17. Johnson, J. 1922. The relation of air temperature to the mosaic disease of potatoes and other plants. Phytopathology 12: 438-441.

18. Kassanis, B. 1952. Some effects of high temperature on the susceptibility of plants to infection with viruses. Ann. Appl. Biol. 39: 358-368.

19. Martin, J. 1978. Procedures for evaluating wheat streak mosaic virus resistance. Plant Dis. Reptr. 62: 1062-1066.

20. McKinney, H. H. 1923. Investigations of rosette disease of wheat and its control. J. Agr. Res. 23: 771-800.

21. McKinney, H. H. and E. E. Clayton. 1945. Genotype and temperature in relation to symptoms caused in Nicotiana by the mosaic virus. J. Hered. 36: 323-331.

22. McKinney, H. H. and R. W. Fulton. 1949. Local susceptibility of cotyledons and leaves of cucumber to tobacco mosaic virus. Phytopathology 39: 806-812.

23. Mckinney, H. H. and W. J. Sando. 1951. Susceptibility and resistance to the wheat streak mosaic virus in the genera Triticum, Agropyron, Secale, and certain hybrids. Plant Dis. Reptr. 35: 476-479.

24. Meiners, J. P. 1979. Differential effect of light and temperature on reaction of snap bean cultivars to peanut stunt virus. Phytopathology 69: 536 (abstract).

25. Moore, J. D. 1967. Relation of temperature to prune dwarf symptom

expression on Italian prune leaves. Phytopathology 57: 823 (abstract).

26. Panzer, J. D. 1958. The effect of pre-inoculation temperature on test plant susceptibility to alfalfa and tobacco mosaic viruses. Phytopathology 48: 550-552.

27. Pfannenstiel, M. A. and C. L. Niblett. 1978. The nature of the resistance of Agrotricums to wheat streak mosaic virus. Phytopathology 68: 1204-1209.

28. Pilowsky, M., R. Frankel, and S. Cohen. 1981. Studies of the variable reaction at high temperature of F_1 hybrid tomato plants resistant to tobacco mosaic virus. Phytopathology 71: 319-323.

29. Pound, G. S. 1953. Relation of air temperature and virus concentration to mosaic resistance in cabbage. Phytopathology 42: 83-88.

30. Pound, G. S. and P. Cheo. 1952. Studies on resistance to cucumber virus 1 in spinach. Phytopathology 42: 301-306.

31. Pound, G. S. and C. Garces-Orejuela. 1959. Effect of photoperiod on the multiplication of turnip mosaic virus in rape. Phytopathology 49: 16-17.

32. Pound, G. S. and L. G. Weathers. 1953. The relation of host nutrition to multiplication of turnip virus 1 in Nicotiana glutinosa and N. multivalis. Phytopathology 43: 669-674.

33. Rex, T. H. 1954. Factors affecting development of necrosis in some bean varieties inoculated with common bean mosaic virus. Phytopathology 44: 508 (abstract).

34. Sameul, G. 1931. Some experiments on inoculating methods with plant viruses, and on local lesions. Ann. Appl. Biol. 18: 494-506.

35. Schneider, I. R. and J. F. Worley. 1958. Effect of high temperature on site and extent of multiplication of southern bean mosaic virus. Phytopathology 48: 244-248.

36. Sebesta, E. E. and R. C. Bellingham. 1963. Wheat viruses and their genetic control. Proc. 2nd Int. Wheat Genet. Symp., Lund, Sweden 1963. Hereditas Suppl. 2: 184-201.

37. Sebesta, S. 1982. An analysis of agronomic characteristics and reaction to wheat streak mosaic virus in four hard red winter wheat crosses. M.S. Thesis, Oklahoma State University.

38. Shaalan, E. G. and W.H. Sill, Jr. 1966. Breeding wheat for

resistance to leaf rust, stem rust, bunt, soil-borne wheat mosaic virus, wheat mosaic virus, and wheat streak mosaic virus. Phytopathology 56: 644-668.

39. Singh, G. P., D. C. Arny, and G. S. Pound. 1960. Studies on the stripe mosaic of barley, including effects of temperature and age of host on disease development and seed infection. Phytopathology 50: 290-296.

40. Silbernagel, M. J. and A. M. Jafri. 1974. Temperature effects on curly top resistance in Phaseolus vulgaris. Phytopathology 64: 825-827.

41. Sill, Jr. W. H. 1952. Effects of various temperatures upon symptomatology of wheat yellow streak-mosaic virus. Phytopathology 42: 474 (abstract).

42. Slykhuis, J. T. 1953. Wheat streak mosaic in Alberta and factors related to its spread. Can. J. Agr. Sci. 33: 195-197.

43. Slykhuis, J. T. 1955. Aceria tulipae Keifer in relation to the spread of wheat streak mosaic. Phytopathology 45: 116-126.

44. Slykhuis, J. T., J. E. Andrews, and U. J. Pittman. 1957. Relation of date of seeding winter wheat in southern Alberta to losses from wheat streak mosaic, root rot, and rust. Can. J. Plant Sci. 37: 113-127.

45. Spencer, E. L. 1935. Effect of nitrogen supply on host susceptibility to virus infection. Phytopathology 25: 178-191.

46. Spencer, E. L. 1935. Influence of phosphorus and potassium supply on host susceptibility to yellow tobacco mosaic infection. Phytopathology 25: 493-501.

47. Staples, R. and W. B. Allington. 1956. Streak mosaic of wheat in Nebraska and its control. Nebraska Agr. Exp. Sta. Res. Bull. 162.

48. Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill, N.Y. 481pp.

49. Stevenson, W. R. and R. E. Rand. 1970. Influence of temperature on symptom expression of pea seed-borne mosaic virus in pea. Phytopathology 60: 1316 (abstract).

50. Stingl, H. and T. H. King. 1965. Influence of temperature on mild mottle and veinbanding in Fragaria vesca and Fragaria virginiana. Phytopathology 55: 919-922.

51. Weathers, L. G. and G. S. Pound. 1954. Host nutrition in relation to multiplication of tobacco mosaic virus in tobacco. Phytopathology 44: 74-80.

52. Yarwood, C. E. 1956. Heat induced susceptibility of beans to some viruses and fungi. Phytopathology 46: 523-525.

53. Zummo, N. 1967. Effect of treatment of seed cane on susceptibility of sugarcane to sugarcane mosaic virus. Phytopathology 57: 83-85.

VITA

Shen-Then Chang

Candidate for the Degree of

Doctor of Philosophy

Thesis: ENVIRONMENTAL FACTORS AFFECTING THE RESISTANCE OF CERTAIN AGROTRICUMS AND THEIR DERIVATIVES AGAINST WHEAT STREAK MOSAIC

Major Field: Plant Pathology

Biographical:

Personal Data: Born in Taipei, Taiwan, Republic of China, January 18, 1952, the son of Mr. and Mrs. Chan-Chi Chang.

Education: Graduated from Chian-kuo High School, Taipei, in June, 1970; received Bachelor of Science in Agriculture degree in Plant Pathology from National Taiwan University in 1974; received Master of Science degree in Plant Science from University of Idaho in 1979; completed requirements for the Doctor of Philosophy degree at Oklahoma State University in July, 1984.

Professional Experience: Research assistant, Department of Plant Pathology, National Taiwan University, 1976; graduate research assistant, Department of Plant Pathology, Oklahoma State University, 1981-1983.